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Agricultural Research

What a Way to Grow!

Story on page 6

Genesis From a Single Cell

Growing plants from a single cell or from tiny bits of plant tissue is a sometimes difficult but almost always rewarding enterprise. This issue of *Agricultural Research* features an article about the advances that tissue culture—as this technique is called—has made both in commercial propagation of plants and in basic scientific research.

Despite increasing knowledge in the field, tissue culturists often say that regenerating plants from cellular tissue nurtured in test tubes and petri dishes is still more of an art than a science.

True or not, tissue culture is a major part of biotechnological research and is a specific subject of scientific inquiry as well as a laboratory technique. Ultimately, it can be seen as bringing together two components of biological research that are critical to the development of better crops: genomic analysis and the study of tissue differentiation.

Genomic analysis deals with the chemical (DNA) structure of chromosomes, which are the tightly coiled strands of genes in the nucleus of a cell. The study of tissue differentiation, on the other hand, asks how these genes are able to coordinate the multiplication of cells into different tissues, organs, and functions that give rise to whole plants. Naturally, these two specialties are closely related, and that relationship is examined through the science of tissue culture.

Tissue culture is based on a 19th century concept of totipotency, which holds that each cell within an organism contains all of the genetic information necessary for creating a new organism. As it applies to plants, the concept has been proven correct. A single plant cell, whether it be taken from a leaf or stem or flower or root, can grow into a whole new plant, provided the cell is properly nurtured in a solution of salts, sugars, vitamins, and hormones.

There is at least one advantage to propagating plants through tissue culture: once a plant with the desired characteristics is found or developed, massive numbers of identical and genetically uniform plants can be produced immediately. As the article on page 6 points out, many horticultural crops are being mass-produced in new industries based on tissue-culture methods.

But attempts at growing many major crops from cells have so far resulted in genetic deviations rather than genetic uniformity. Specifically, there has been an unpredictable loss of hereditary traits in the regenerated plants. Known as somaclonal variation, which means the changes occur without a sexual mix of chromosomes, this seeming contradiction to genetic logic has inspired new research into the use of tissue-culture technology. It may well prove to be a way to induce mutations that can be tracked from their inception at the molecular level.

Once a mutation is established, it can serve as a "marker" for studies on the genetic control of tissue differentiation. By learning how mutations of DNA are expressed during plant growth in tissue culture, researchers can acquire the knowledge necessary to make alterations of the genetic code more systematic and the outcomes more predictable. Similarly, the technology of tissue culture will also be refined to make it a reliable method for the genetically stable propagation of our major crops.

Such declarations may sound premature. There is no guarantee that one research development will lead to another, or that we will acquire the necessary knowledge every step of the way. But a strong program of systematic experimentation in tissue culture will increase the likelihood of success.

Jerome P. Miksche, National Program Leader for the Agricultural Research Service in plant physiology and biotechnology, says the key to such a program will be the development of model tissue-culture systems that can be physically, chemically, and biologically regulated to yield results that are predictable and reproducible. The models must establish complete control of all tissue-culture conditions and be able to relate those conditions to changes in DNA. Only then, he says, can we begin to truly understand the processes of somaclonal variation and tissue differentiation in plant growth.

Miksche believes that a concerted effort in the development of model tissue-culture systems will enable us to achieve, over the next 10 to 15 years, a high degree of genetic stability in all crops propagated through tissue-culture techniques. Without such an approach, he says, the timetable could be as long as 50 years. If so, the decades that are saved could mean a lot to agriculture by the turn of the century.—**Steve Miller,** ARS.

Agricultural Research

This strawberry plantlet is but one example of numerous horticultural crops being lab-cloned commercially through a technique called tissue culture. Story on page 6.
(0386X429-25A)

David F. Spencer was incorrectly identified in a photograph on page 15 of the March 1986 issue.

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Waxy Leaves Are Healthy Leaves

Homemakers may not like it on their floors, but waxy buildup could be healthy for plants and animals.

Research has shown that natural wax on the surface of plant leaves slows the loss of moisture from a plant. More recently, scientists have learned this waxy coating acts as a barrier to insect attacks.

Agricultural Research Service scientists in Logan, UT, and Manhattan, KS, are breeding new waxy-leaf alfalfas to take advantage of these characteristics.

In addition, "wax may reduce the risk of bloat in cattle that graze on highly nutritious alfalfa plants," says plant geneticist Melvin D. Rumbaugh at Logan.

Bloat is a frequently fatal accumulation of gas bubbles in an animal's stomach. Cattle are the most likely to bloat, but sheep and other ruminants can be affected. Research with plants other than alfalfa indicates that wax on plants helps break down the foam, allowing the gas to escape, and that the more wax, the less bloat.

Rumbaugh estimates losses due to bloat reach $300 million a year. "It's even more," he says, "when you account for milk and weight losses that result when farmers try to reduce bloat by feeding their livestock less alfalfa."

Rumbaugh and colleagues at Logan found that the natural wax on 28 common types of alfalfa varies considerably—from 138 to 460 milligrams per square meter of leaf surface.

Rumbaugh has developed both high-wax and low-wax alfalfas by crossing these U.S. varieties. They will be compared during the 1986 growing season for their ability to withstand drought and insect attacks.

Agronomist Edgar L. Sorensen, in cooperation with the Kansas Agricultural Experiment Station at Manhattan, is taking a different route for waxy leaves. He crossed a wild, low-growing waxy species from northern Italy with a widely used U.S. species. The Italian alfalfa, *Medicago prostrata,* typically grows only inches long and flourishes in arid areas.

Wax production of this hybrid varies with the amount of water available to the plant. On plants grown on dryland, wax production increased and many strumae—balls of wax— developed on the leaf surface. None developed on irrigated plants.

Says Sorensen, "These drought-resistant characteristics can be bred into commercial alfalfa varieties, making it possible to grow alfalfa in dry areas where presently available varieties cannot survive."—By **Linda Cooke-Stinson** and **Howard Sherman,** ARS.

Melvin D. Rumbaugh is at the USDA-ARS Forage and Range Research Unit, Utah State University, Logan, UT 84322. Edgar L. Sorenson is in USDA-ARS Plant Science Research, Department of Agronomy, Throckmorton Hall, Kansas State University, Manhattan, KS 66506. ■

Eggs of Cotton Pest Devoured

Voracious insects that thrive naturally in the southwestern United States can devour up to 98 percent of eggs laid by the pink bollworms in cottonfields.

"At least three insects—green lacewings, lady bird beetles, and collops beetles—might be reared and released to protect cotton crops early in the season," says Thomas J. Henneberry, an Agricultural Research Service entomologist at Phoenix, AZ.

The highly destructive pink bollworm causes annual losses estimated at 25 percent of the crop value in the southwestern U.S. cotton-growing area.

"Most predatory insects seem to have insatiable appetites.

Although delicate and beautiful as an adult, in its larval stage the green lacewing *(Chrysopa carnea)* can be a voracious predator of the pink bollworm. (Photo by Grant Heilman Photography)

Unfortunately, they are in constant battle to survive and often eat each other before attacking the pink bollworm," says Henneberry.

"Our goal is to find the one or two hungriest predators that would feast on pink bollworm eggs before eating each other. Then we could develop methods to expand their populations in cotton-growing areas," says Henneberry.

Henneberry doesn't believe his research will lead to complete elimination of insecticides. However, he does believe natural predators might be manipulated or conserved to provide control early in the growing season so fewer chemical applications will be needed later to reduce damage.

"If we can eliminate even one insecticide application, we reduce potential risk to the environment and make cotton production more efficient," says Henneberry.

He says none of the beneficial insects he has studied damage any commercial crops—only other insects.

Pink bollworms are most susceptible to predators early in the cotton-growing season. It is during this time that young plants provide few hiding places for their eggs.

Subsequent pink bollworm generations—there can be up to 5 generations in one 9- to 10-month

growing season—lay eggs on cotton plant parts that are more inaccessible to predators.—By **Dennis Senft,** ARS.

Thomas J. Henneberry is at USDA-ARS Western Cotton Research Laboratory, 4135 East Broadway, Phoenix, AZ 85040. ■

Greenbugs Fooled and Leashed

How do you thwart a greenbug that can fly hundreds of miles and loves to feed on growing wheat, grain sorghum, and a host of other crops? One way, according to Agricultural Research Service entomologist Robert L. Burton, is to turn its genetic guidance system against it.

It seems that when greenbugs—really aphids—decide to migrate, they fly toward open sky. After a while their instinct reverses and they fly toward darker areas—bare soil, green crops, etc.

Gardeners have known for years that aluminum foil between vegetable rows cuts aphid infestations to a fraction. Aluminium foil isn't practical for wheat fields in the Great Plains, of course, but new research at Stillwater, OK, shows that straw, stalks, and leaves from a previous crop left on the soil surface work the same way. "Plowing under crop residues after harvest is a mistake when it comes to greenbugs," Burton says.

Another way to avoid the greenbug menace is to find varieties of wheat better able to withstand greenbug attacks. With this in mind, Oklahoma State University agronomist Jim Ryan, in cooperation with ARS entomologist James A. Webster, has taken to attaching a "leash" to the pests.

Obviously, it takes a mighty small "leash" for a pest not much larger than the period at the end of this sentence. The researchers actually glue a fine gold wire—finer than a spider's silk—to the backs of greenbugs.

With the aid of a magnifying glass, a tiny drop of silver glue is suspended on the end of a thread. A greenbug is gently touched to the glue drop, leaving a small spot on its back. The end of the gold wire is placed in the drop and the insect is then properly leashed.

The opposite end of the gold wire is soldered to a copper wire, which is buried in moist soil containing the wheat plant on which the harnessed insect is sitting. The result—a complete electrical circuit whenever the insect takes a "bite."

Ryan explains that very low voltages of electric current pass through the insect and plant with no harm to either. But with the use of an amplifier, these voltages are brought back to measurable levels and recorded.

The real goal is not to take the insects out for exercise, Ryan says, but to learn more about their feeding behavior and why and how some wheat plants resist them.

The researchers say that at least two of the wheat lines being studied show some tolerance to greenbug feeding.—By **Lloyd McLaughlin,** ARS and **Fred Causley,** Oklahoma State University.

Robert L. Burton is in USDA-ARS Plant Science Research, P.O. Box 1029, Stillwater, OK 74076. ■

Rare Bacterium Helps Cattle Digest Toxic Plant

Popular folklore has it that goats will eat nearly anything. So it may not seem surprising that goats in Hawaii have been eating *Leucaena*—a sometimes poisonous plant—and getting away with it.

Leucaena grows as a small, bushy tree. It is often praised for its drought resistance and its nutritional qualities as livestock feed.

Because the plant could become a valuable forage crop if other animals were immune to its toxic compounds, Agricultural Research Service scientists are studying how the goat survives its effects.

The goat gets its resistance from a special bacterium in its stomach that protects it from toxic substances in the plant, says Milton J. Allison, a microbiologist at the agency's Animal Disease Center, Ames, IA.

Tests of cattle in Texas and Iowa indicate they don't have the same natural protection. Allison and biological technician Herbert M. Cook at Ames compared stomach fluids in goats and cattle in the two states and found that cattle lack the protective bacteria that goats have.

But the bacteria can be transplanted from one animal to the other, as demonstrated in studies by Raymond J. Jones, of the Australian Commonwealth Scientific and Industrial Research Organization.

In Australia, steers with *Leucaena* poisoning symptoms—loss of hair, goiter, and ulcers of the esophagus—soon recovered after being fed mixtures of bacteria from food digesting in the stomachs of Hawaiian goats. In fact, other animals in the test herd recovered without the treatment, indicating that the beneficial bacteria were transmitted from animal to animal.

The plant's toxicity comes from a compound called 3-hydroxy-4(1H)-pyridone (DHP) that is produced by enzymes in both *Leucaena* leaves and rumen contents from an amino acid called mimosine. A pure culture of a bacterium that degrades DHP has recently been isolated by Allison and Cook from the mixed bacteria found in the goats. This, as yet, unidentified bacterium appears to be different from any previously described species.

Milton J. Allison is at the USDA-ARS National Animal Disease Center, P.O. Box 70, Ames, IA 50010. ■

Assembly Line Plants Take Root

Biologist Peter Carlson, of Crop Genetics International, checks growth characteristics of disease-free sugarcane plants. (0286X282-18)

Five years after Peter S. Carlson helped start Crop Genetics International, a small high-tech company in Dorsey, MD, he is ready for sales—of laboratory-cloned sugarcane and apple trees. As far as he knows, his company is the first in this country to sell tissue-cultured sugarcane and apple tree varieties.

"Plant tissue culture" is a term for a variety of techniques used to grow or genetically modify, preserve, or study plants and plant parts in laboratories, from tissue or even a single cell.

"Tissue-culture propagation is more an art than a science for commercial growers," says Carlson, a pioneer in tissue-culture research who has spent the past 5 years mastering its practical use. For the purposes of his company he needs to transform tissue culture from a science to an easy-to-learn production skill.

Carlson began with a few sugarcane plants raised in disease-free, quarantine conditions at USDA's Agricultural Research Service facility in Beltsville, MD, about 15 miles southwest of Crop Genetics. A. Graves Gillaspie, a plant pathologist with the ARS Microbiology and Plant Pathology Laboratory at Beltsville, provided the first few plants, at the request of Crop Genetics. ARS offers this type of service to any private or public firm that asks, in an effort to put publicly funded research to the widest possible use.

From a Few to Many

"Plantlet" is a word used by horticulturists to describe tiny plants that develop from their parents vegetatively, without seed.

But, Carlson's plantlets give a new twist to that definition—they come from pencil-sized tissue taken from near the tops of sugarcane stalks. He sterilizes the tissue surface and then puts these small stalks in a gelled solution (medium) in a petri dish. The medium causes the original tissue to sprout sugarcane plantlets. And more and more and more.

In 3 months, Carlson can get many hundreds of plants from one piece of stalk.

As the tissue multiplies, lab technicians, called culturists, must separate the plants into more containers and change the solutions. When the plant is ready to root, it's put in a larger container with the medium altered to cause rooting instead of sprouting. Once it's rooted, the plantlet goes into the greenhouse where it's planted in potting soil in Styrofoam trays.

If these were apple trees, they'd be ready for sale to an orchard. But sugarcane farmers don't plant sugarcane seedlings or even plantlets. They use either whole sugarcane stalks or stalk pieces (seed pieces) in furrows, growing plants from the buds on the stalks. In this sense, a sugarcane stalk is a lot like a potato: Both are stems with a large number of buds, the potato being an underground stem or tuber.

To get these seed pieces from sugarcane, Carlson ships his plantlets to company farms in Florida and Louisiana, close to sugarcane country but not close enough to risk disease contamination from sugarcane fields. There the plantlets are grown just the way farmers grow sugarcane for seed. It's these stalks or seed pieces which farmers buy.

Why should farmers buy stalks when they can grow their own? "For the same reason potato farmers have been doing so for three-quarters of a century: They can make more money. We use the latest technology to give them disease-free stalks that are more vigorous and more productive than what they now grow," Carlson says.

Normally, tissue-culture propagation of plants is only cost effective for plants that are individually valuable such as apple trees or orchids. Carlson admits that his sugarcane plantlets are the most expensive sugarcane plants in the world. That's because of the number of tissue culturists Carlson has to pay to give the plants tender loving care.

But Carlson says tissue culture gives him such high-yielding sugarcane that it offsets the labor costs. This ability of tissue culture to grow plants that are more vigorous and more productive and have better growth characteristics is generally true for all plant tissue culture. Carlson and other scientists suspect it may have something to do with the growth hormones used in the medium.

Gillaspie says he's not sure whether that vigor will last from one crop to the next, but it doesn't matter because just eliminating disease is enough to do wonders for yields.

"Carlson's doing for sugarcane what was done for potatoes many years ago—producing seed pieces certified to be essentially disease-free," Gillaspie says. "The only difference is that he's doing it with tissue culture." Gillaspie is referring to the state programs designed to produce healthy potatoes, the first begun by the University of Wisconsin just before 1920.

Carlson—like others—has benefited from ARS research nationwide and the desire of the agency to share its experience with the public. For sugarcane, he looked at the agency's work being done in cooperation with the American Sugar Cane League, the Florida Sugar Cane League, and the Hawaiian Sugar Planters' Association.

For tissue-cultured apple trees, Carlson went to Richard H. Zimmerman, a plant physiologist at the ARS Fruit Laboratory in Beltsville. For the past several years, visitors to the research center have marveled at the sight of his laboratory-started apple trees growing on the slope of a hill overlooking rows of greenhouses. Zimmerman says an important task he and other ARS scientists face is to find out how apple trees and other tissue-cultured plants do in the real world outside the lab. "With trees you just can't see flowers and fruits

A tiny apple tree produced from tissue culture is held by plant physiologist Richard Zimmerman. (0286X275-12A)

in 1 year. It takes several years. Only then can you start testing, and you must test over a number of years—a minimum of 10," Zimmerman warns.

Zimmerman planted his first tissue-cultured apple orchard in Beltsville in 1979 and added trees during the following 5 years. In the past few years, he has been sending trees throughout the United States for testing, even sending some to Canadian government agricultural researchers.

His oldest apple trees have grown outdoors for just 7 years, not enough time for a verdict. And peach trees only began to move outdoors a year ago, at Beltsville.

On the other hand, Zimmerman says that strawberries and thornless blackberries—because of the much

Assembly Line Plants Take Root

Horticulturist Olivia Broome examines cloned strawberries in growth chamber. (0286X238-6)

shorter time in which they mature and bear fruit—have been sufficiently field-tested.

Zimmerman agrees with Carlson that tissue-culture propagation is both an art and a science. "There's a lot of variability within species as well as between species. Take blueberries as just one example. Some cultivars are very easy and others are very difficult to grow with tissue culture. We don't know why."

Well aware of the risks as well as the rewards, Carlson is planning for success. For the near future, he is looking at the tissue-culture system developed for peaches by Freddi A. Hammerschlag, a plant physiologist with the ARS Tissue Culture and Molecular Biology Laboratory at Beltsville. Hammerchlag says that trees and shrubs are much more demanding to propagate than other plants. As one example, she found her peach tree plantlets refused to cooperate without a dose of "winter" each year—6 weeks in a refrigerator.

Mutations Are Both Good and Bad

But Hammerschlag has moved beyond propagation to focus on using tissue-culture techniques to search for peach plants that are more resistant than currently cultivated varieties to bacterial leaf spot disease. The disease is a major problem to East Coast peach growers because the bacterium that causes the disease thrives in humid summers and covers the fruit with black spots. It causes the leaves to drop and eventually kills the trees.

Tissue culture enables Hammerschlag to select plant cells rather than whole plants for disease resistance. She uses callus, tissue which is formed to heal wounds and is made up of cells that have not yet organized to form tissues and plant parts.

Hammerschlag uses callus because the unorganized cells frequently mutate in the growth medium. Mutations mean variety and that's what she and plant breeders work with.

The potential of tissue culture to induce mutations, an ability not yet understood, can be a problem when propagating a variety for commercial use. For example, a Delicious apple must be red with five lobes on the blossom end and have an overall elongated shape. Lose one of those characteristics and you don't have a Delicious apple.

Olivia C. Broome, an ARS horticulturist who grows tissue-cultured strawberries at the Beltsville Fruit Laboratory for researchers worldwide, says as long as tissue-culture

High-Tech Disease Detection

"Just because a plant is produced from tissue culture does not necessarily mean it is disease free," says Roger H. Lawson, research leader of the Florist and Nursery Crops Laboratory at Beltsville, MD.

Using the latest methods to detect disease, plant pathologists Ramon L. Jordan and John Hammond look into plant tissue for two of the smallest known disease-causing agents (pathogens): viruses and viroids. Viruses are infectious agents consisting of a segment or segments of nucleic acid surrounded by a protein coat. Viroids are smaller segments of RNA (ribonucleic acid) without a protein coat.

"Most virus and viroid diseases do not kill an infected plant but may make it unsalable by stunting its growth or damaging its fruit, flowers, or leaves," Lawson says. "The whole idea of producing tissue cultures that are pathogen free is to improve

propagators are careful, they probably won't have to worry about mutations. In addition, the mutation threat is greatly reduced when propagators start with stable, organized tissue such as plant shoot tips rather than callus or cells.

Broome's work begins in the greenhouse, where she cuts off the ends of strawberry runners before they can fully develop into new plants. She takes the runner tips to her lab where the sterility begins with a bath in a mild household bleach and detergent solution.

Broome runs a lab whose sterility is only a little less than that of a hospital operating room. Her "operating room" is sterilized with ultraviolet light whenever it is unoccupied. When the door is opened, positive pressure from air flowing through large filters near the ceiling keeps any contaminants from entering. In one hand she holds a heat-sterilized surgical scalpel; in the other, a jeweler's forceps, sterile and sharpened specially to grasp the minute particle of living tissue she will remove for culturing.

Under a binocular microscope designed to aid dissecting, set to magnify 10 to 30 times, she begins to cut away at the living tissue. Aided by a measuring scale visible in one eyepiece, she carefully slices very thin cross sections, carving away unwanted tissue, until she reaches the meristem tip, the growing point of the runner.

When Broome finds the tip, she uses forceps to pick it up and very gently plant it top side up on the translucent medium in a jar, careful that it doesn't sink in and suffocate. Sitting on the medium, the tip looks like a grain of salt. The medium and the jar had previously been heat-sterilized.

The medium's recipe includes small amounts of the essential minerals found in fertilizers used by home gardeners. But Broome also adds other minerals, vitamins, sugar, and the all-important growth regulators, which are synthetic duplicates of natural plant growth hormones. A cytokinin hormone is used to induce the tissue to grow shoots, while an auxin hormone is used later to induce the formation of roots.

Sugar feeds the growing plants since they have neither sufficient light to photosynthesize nor roots to draw in nutrients—both needed to make food sugars in nature. The sterility ensures that the medium doesn't end up raising a bunch of fungi or bacteria as well.

crop quality and productivity."

One method that has been used by Jordan and Hammond and other Agricultural Research Service scientists to detect viruses and viroids is a technique called nucleic acid spot hybridization. Nucleic acids make up the genetic code for amino acids, the building blocks of proteins. And hybridization, Jordan explains, is the "joining of matching sequences of genetic material coming together like a zipper."

The zipper analogy is a good way to explain how genetic codes can be used to detect viruses or viroids in plants.

The genetic zipper has four kinds of teeth that make up a nucleic acid. When the DNA (deoxyribose nucleic acid) zipper splits, it leaves a strand of exposed teeth. A new RNA-strand of teeth matches up with the exposed teeth, but each tooth fits only with a complementary tooth. This strand of matching RNA is the code for selecting the strings of amino acids which make up proteins.

Since nucleic acid sequences are usually constant among specific pathogens, says Lawson, they can be used to identify specific nucleic acids in viruses and viroids. To detect these pathogens, a known virus' or viroid's RNA is used to make a strand of complementary DNA (cDNA) that will be able to recognize viral (or viroid) RNA in a plant-tissue extract.

Tissue extracts from a suspect plant are spotted on a thin membrane filter. The nucleic acid in the tissue extract is then immobilized by baking the filter. The filter is soaked in a solution containing the cDNA, which will hybridize to any complementary sequences of nucleic acids it finds. The cDNA and RNA of identical or related viruses and viroids "zip" together.

After the filter is washed to remove any unattached cDNA, the cDNA-RNA hybrids show up as dark spots on x-ray film that has been placed against the filter, because the cDNA has previously been made radioactive. A dark spot indicates that the virus or viroid is present in that tissue extract.

One way Jordan and Hammond use this technique is to detect and compare viruses infecting fruit and bulb crops. Another very important use of hybridization has been to routinely check seed potato lots for the presence of the potato spindle tuber viroid.

This specific application of the technique was developed and is used by Robert A. Owens and T.O. Diener, both with the ARS Microbiology and Plant Pathology Laboratory in Beltsville, and Luis Salazar with the International Potato Center in Lima, Peru.

Hybridization is one of the few techniques that can detect viroids, because currently used serological methods of virus detection rely on the protein coat which is absent in viroids.—By **Deborah Aksler,** ARS.

Roger H. Lawson, Ramon L. Jordan, and John Hammond are at the USDA-ARS Florist and Nursery Crops Laboratory, Bldg. 004, Room 101, BARC-West, Beltsville, MD 20705. ■

Assembly Line Plants Take Root

After the operation, the jar is placed on a shelf with many others, in a well-lit area. Temperature and length of light each day are carefully controlled. In 6 to 8 weeks, each tip grows into a strawberry plantlet. Then each plantlet is cut up and in 3 or 4 weeks, each piece will grow anywhere from three to eight more plants. Before the next month is up, each of those plants will again be cut up into pieces. And so on, every few weeks.

Plantlets grown from small meristem tips are usually virus free. There are several theories to explain this. One is that the meristem tip itself is virus free because it's isolated from vessels that could spread the virus. Another theory says the meristem tip may not be virus free but there may be something in the medium that acts as an antiviral agent. Or the meristem tips may grow so fast they starve any viruses that might have been present.

But beginning with disease-free plants isn't enough. Tissue culturists must keep the tissue and resulting plantlets free of disease as well. Broome advocates testing the first batch of plantlets for disease before mass-producing them.

While tissue culture doesn't guarantee disease-free plants, it gives growers the best chance to keep healthy plants healthy.

The propagation of horticultural plants is the main commercial use of tissue culture worldwide, because individual horticultural plants are so valuable. They're also most often vegetatively propagated through cuttings or grafts rather than grown from seed. Anyone who buys house plants such as the tropical ferns sold in supermarkets is probably buying a plant that got its start in a lab.

There are more than 200 tissue-culture labs in the nation that grow horticultural plants, with a total estimated production last year of 60 to 65 million plants. The potential for this production to grow is tremendous, considering the fact that in the past 2 years, several labs have expanded their production capacity to 15 or 20 million plants a year. This is more than the market can currently bear, so the development of new markets is a high priority.

Geneticist Brent Tisserat (left) and chemist Carl Vandercook check differences between growth of carrots in test tube and those in jar where growth medium is changed automatically without disturbing plantlets. (0985X987-20)

Tissue culture may well replace most forms of vegetative propagation, especially grafting, which Zimmerman describes as a "miserable job; it's hard to find people trained and willing to do it."

Grafters work in the hot summer sun, in pairs over a large field—one stooping or kneeling or crawling to make a cut or two in each rootstock and insert a bud in each cut, the other following to bandage the graft. The grafters usually use two buds per rootstock to double their chances of success. Even so, some will not grow into trees.

A tissue culturist works indoors and can turn one bud into hundreds of thousands of trees, each with their own roots. And if costs are kept down, each tree should be cheaper.

The biggest thing keeping costs up—and therefore holding tissue-culture propagation back—is the amount of labor needed to run a tissue-culture lab.

Automation to the Rescue

Automation is one solution to the problem of high labor costs. ARS scientists in California have found a way to automate their tissue-culture work and at the same time achieve at least two to four times the growth rate of conventional tissue-culture systems.

Plant geneticist Brent Tisserat and chemist Carl E. Vandercook use a computer to control the flow of liquid nutrients to plantlets. The nutrients flow through a network of tubing to plastic boxes or glass jars that house the growing plants. Tiny glass beads line the bottoms of these

containers to keep the upper part of the plants above the solution.

Every 2 hours, all of the nutrient solution is automatically pumped out of the boxes into storage jars. The glass beads help the roots air out before the nutrient solution is cycled back to the plants.

Once a day, a small amount of the recycled solution is automatically drained from the storage jars and an equal amount of fresh solution is added.

"Our experiments with orchids, date palms, citrus, and other plants have consistently produced outstanding growth, typically two to four times that of conventional tissue-culture systems," Tisserat says.

"The most dramatic growth has been with carrots, which traditionally do very well in tissue culture. With the automated system, they grew up to 15 times faster."

Besides speed, there is less risk of damage to tiny plantlets than with conventional techniques because there are no transfers. Tisserat explains: "Normally, plants have to be trimmed, subdivided, and moved to fresh nutrients in new test tubes at least every 2 to 8 weeks. With the automated systems, plantlets don't have this stress. They stay in one place until they are ready to be planted in pots or beds."

For Tisserat and Vandercook, the new system has a special advantage: They can keep plants—and plant parts—alive and growing for months longer than in traditional tissue culture. That's important, because they need to be able to follow subtle changes in the chemical composition of a plant as it grows.—By **Don Comis** and **Marcia Wood,** ARS.

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Living Plant Libraries

Scientists throughout the world are turning to tissue-culture techniques to preserve important plant varieties.

At the Agricultural Research Service National Clonal Germplasm Repository in Corvallis, OR, scientists are using long-term storage of tiny bits of plant tissue for a backup to field plants and potted plants that could be destroyed by disease, drought, or other disasters.

Repository director Harry B. Lagerstedt says plants in the field or in pots take up a lot of space and are expensive to maintain: They have to be watered, pruned, sprayed, fertilized, heated in the winter, and repotted every year or two.

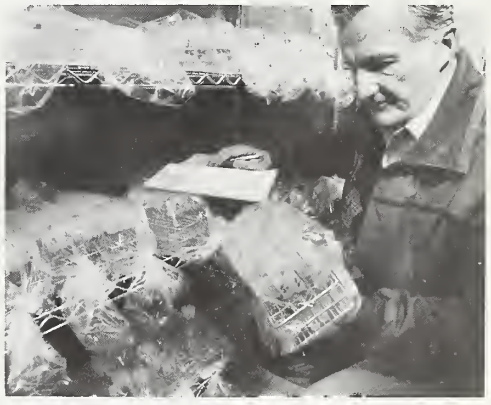
He says plants that would ordinarily fill a greenhouse can be stored in test tubes in one freezer-sized chamber. Cold storage for long periods allows living plants to perform more like seeds.

"A seed is a highly efficient way to store a plant," says Lagerstedt. "It's small, and storing it takes little or no maintenance. So it is with tissue culture."

While carefully manipulating temperature and light, the scientists devise recipes that dramatically slow the growth of plants. Lagerstedt says mint and strawberry tissue cultures in test tubes have been tucked away in refrigerators—at minus 1°C—for up to 2 years. When they were removed from cold storage, the plants resumed normal growth.

Scientists at the repository have also been freezing tissue, says Lagerstedt. "We take a flyspeck-sized piece of the meristem—the growing point of the plant—slowly freeze it to minus 30° or 40°C, and then drop it into liquid nitrogen at minus 196°C. At this temperature, a plant's life processes are so reduced their state resembles suspended animation."

One problem scientists have is getting plants to begin growing again after they've been stored in liquid nitrogen. "Although we're still in the experimental stage," says Lagerstedt, "our scientists at the repository are the first anywhere to have successfully regrown raspberries and blueberries that had been frozen in liquid nitrogen."

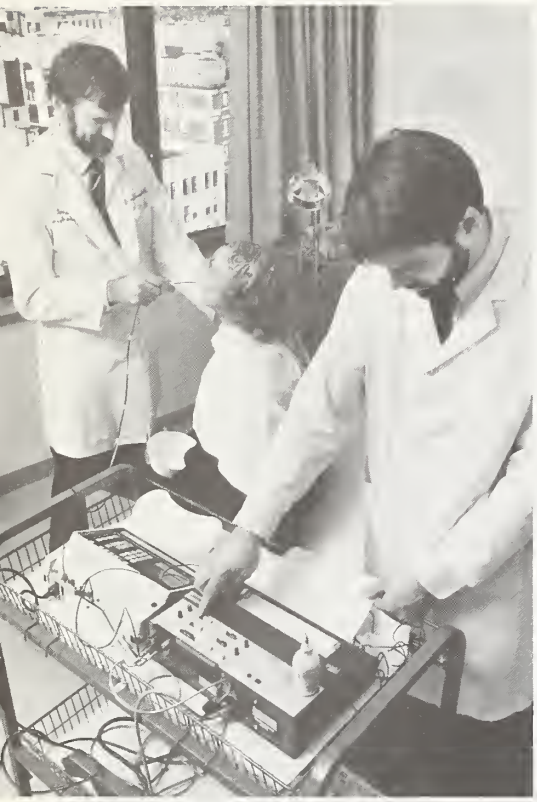


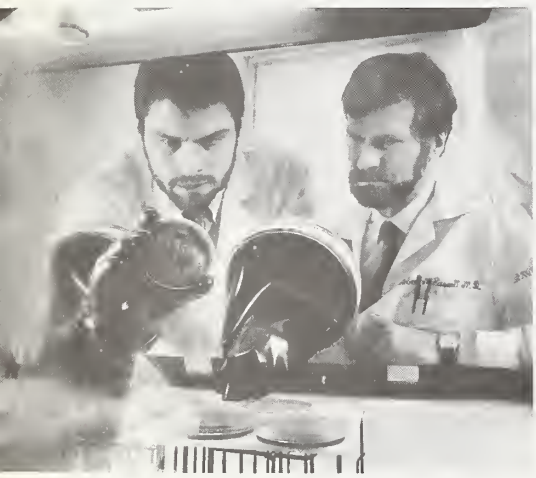
In a cold storage room at the National Clonal Germplasm Repository in Oregon, Harry Lagerstedt inspects tissue-cultured mint, strawberry, blueberry, and raspberry plants. (0286X271-29A)

But their greatest triumph in storing and reviving small fruits has been with strawberries—a 90-percent success rate! Because of this, Lagerstedt and colleagues have developed a 100-year-long experiment for storing strawberry meristems.—By **Howard Sherman,** ARS.

Harry B. Lagerstedt is at the USDA-ARS National Clonal Germplasm Repository, 33447 Peoria Road, Corvallis, OR 97331. ■

Elderly Cautioned on Overuse of Antibiotics, Antacids





Top: In research on the relationship of antibiotic and antacid use to anemia in the elderly, gastroenterologist Robert Russell samples fluid from a volunteer as nutrition biochemist Stephen Krasinski monitors stomach acid levels. (0286X124-4)

Above: Colonies of intestinal bacteria cultured in a climate-controlled chamber are counted by Krasinski and Russell (right). (0286X127-19)

Certain elderly people could suffer from anemia if they use antibiotics for prolonged periods or frequently take antacids at mealtime.

Recent findings indicate that both medications can reduce the body's uptake of folic acid—an essential water-soluble vitamin that aids in red blood cell production, says Robert M. Russell, M.D., acting director of USDA's Human Nutrition Research Center on Aging at Tufts University in Boston. The center is one of five Agricultural Research Service facilities commissioned to study dietary needs in people.

Antacids taken at mealtime simply neutralize the stomach acid necessary for absorption of folic acid, also known as folate or folacin.

Antibiotics, on the other hand, kill off intestinal bacteria that synthesize folic acid in the intestinal tract for people who can't absorb it from food. These people don't secrete enough stomach acid, which normally moves along with food into the upper intestine, providing the proper environment for the absorption of folic acid.

A significant number of elderly people have lost the cells in the stomach that secrete acid. The number ranges between 10 and 50 percent depending on which study is cited, Russell says. In his recent study in the Boston area, 20 percent of the over-60 population had this degenerative condition known as atrophic gastritis.

"The older a person is, the more likely he or she is of having atrophic gastritis," says Russell. A pepsinogen test—performed on a blood sample—is quite accurate in detecting this condition, but it is not commonly used in medical checkups.

"Our studies show that intestinal bacteria compensate for the loss of stomach acid and prevent the body from developing a folate deficiency," Russell says. The bacteria flourish in a nonacid environment and produce folic acid in amounts large enough to override the absorption problem, he explains.

Antibiotics, especially those that kill a wide range of bacteria, may destroy this backup system. "This could precipitate folic acid deficiency within weeks," says Russell.

Deficiency can cause anemia similar to pernicious anemia—the result of vitamin B12 deficiency. Symptoms include fatigue; shortness of breath; a sore, red, smooth tongue; and disturbances of the gastrointestinal tract such as diarrhea.

Russell says the findings also suggest that antacids can cause absorption problems if they are frequently taken with meals. Like atrophic gastritis, antacids reduce the acid environment the body needs to absorb folic acid from foods. However, since the nonacidic condition is temporary, folic acid-producing bacteria don't compensate as they do in people with atrophic gastritis.

Frequent use of antacids could lead to folic acid deficiency in any age group, Russell says, but this practice is more prevalent among the elderly.

Russell says there is evidence that the loss of stomach acid may also reduce the body's uptake of vitamin B12 and calcium. He and colleagues at the USDA center are currently studying this issue.

"If 20 percent of our elderly have atrophic gastritis," he says, "we need to know how it affects absorption of vitamins and minerals. If it impairs absorption, then the dietary requirements would be higher for this group."—By **Judy McBride,** ARS.

Robert M. Russell is with Tufts University at the USDA Human Nutrition Research Center on Aging, 711 Washington St., Boston, MA 02111. ■

Natural Neem Kills Cockroaches and Greenhouse Pests

In his Beltsville, MD, greenhouse, entomologist Hiram Larew measures neem tree seedlings to determine rate of growth. (0386X401-16)

Leafminers left telltale evidence in this lima bean leaf. Maggots burrowed tunnels between the leaf's upper and lower surfaces, reducing photosynthesis. (0386X400-22)

Neem, a stylish tree of Asia and Africa, has yielded a new kind of insecticide for the United States.

Neem products killed or repelled insects on flower and nursery crops when sprayed on leaves or applied to potted plant soil, says Agricultural Research Service entomologist Hiram E. Larew.

Also, six common types of cockroaches were killed by neem-laced baits in other experiments by ARS entomologist Victor E. Adler.

"For centuries, neem leaves and seeds have helped people control insects in the Tropics," says Larew. "Now we're finding ways to use neem in U.S. agriculture."

As natural products, neem insecticides are probably biodegradable and environmentally safe for many uses, although only one has been approved by the U.S. Environmental Protection Agency for insect control on some horticultural crops.

In ARS tests by Larew, the product, called Margosan-O, controlled up to 95 percent of leafminers, a pest costing flower and vegetable growers over $15 million per year in California alone. Neem-based products for cockroach control are not yet approved by EPA, Adler says. However, in experiments he found that both Margosan-O and

Natural Neem Kills Cockroaches and Greenhouse Pests

another lab-tested formula killed young cockroaches and limited egg laying by adults.

Larew says that research by USDA and other agencies in the late seventies showed that neem could control over 80 major insect pests. The work sparked wide interest by scientists in this country and abroad to develop neem products as possible replacements for some synthetic insecticides.

USDA's earlier studies showed that Japanese beetles would rather starve than eat some of their favorite plants that had been treated with neem, according to scientists at the ARS Japanese Beetle Research Laboratory at Wooster, OH.

When added to soil, neem compounds enter roots and move into plant leaves, making plants poisonous to leafminer worms, Larew says. The compounds interfere with the life cycle by somehow jamming hormonal signals for molting. The worms die trapped within their own skins.

Tropical neem trees are scarce in the continental United States but thrive throughout the Caribbean and could do well in southern Florida and Hawaii, according to ARS' Robert J. Knight. Neem, because of its insecticidal properties and high-quality oils, could be grown as a profitable crop by Caribbean farmers, suggests Knight.

About 50 neem trees are growing at research stations in South Florida, Knight says. Over 200,000 have been planted in Haiti in recent years to beautify public roadsides.

In tropical Asia and Africa, neem trees serve many purposes. The wood is used for timber. Leaves are used medicinally and as animal feed; bark is used to tan goat skins; leaves, small branches, and oilseed cakes become fertilizer. Twigs are even used for dental hygiene.

Perhaps neem is most useful, though, for fighting insects. In India, Adler says, ground neem seeds are commonly added to stored grain to keep out insects.

Among the 80 insect pests inhibited by neem extract in research are Mexican bean beetles, Colorado potato beetles, North American grasshoppers, tobacco budworms, carpet beetles, striped cucumber beetles, confused flour beetles, milkweed bugs, citrus mealybugs, and navel orangeworms.—By **Stephen Berberich,** ARS.

Left, top: Neem-based insecticide is sprayed on a chrysanthemum to test its efficacy against leafminers. (0386X400-11)

Left: In India, there are commercially available products incorporating neem extracts. The fact that neem leaves have been used medicinally by people for centuries in India where the tree is indigenous suggests low toxicity for people and other mammals. (0386X403-7)

Above: Mature neem tree. (1178X1571-5)

Hiram Larew, USDA-ARS Nursery and Florist Crops Laboratory, B-470, and Victor E. Adler, USDA-ARS Insect Chemical Ecology Laboratory, B-476, are at the Beltsville Agricultural Research Center, Beltsville, MD 20705. Robert J. Knight is at the USDA-ARS Subtropical Horticulture Research Station, 13601 Old Cutler Rd., Miami, FL 33158. ■

Embryo Rescue Saves New Gene Combinations

By combining two genetic engineering feats—embryo rescue and chromosome doubling—a team of Agricultural Research Service scientists at Logan, UT, have created new plants not seen before in nature.

Geneticist Richard R-C. Wang and colleagues have already produced a new drought-resistant wheatgrass for western rangelands that yields 25 percent more livestock feed.

"With these techniques, almost any combination is possible," says Wang. The same strategy used for crested wheatgrass can be applied to other range grasses and even new wheat varieties. Hybrid embryos—formed by crossing species—are an avenue for transferring desirable genes among related plants.

Hybrids seldom occur naturally in the wheat plant family because the endosperm—the starch portion of the seed that nourishes the hybrid embryo—fails to develop.

"To ensure the embryo's survival," says Wang, "we cut part of the endosperm away from the underdeveloped embryo. Then, we artificially feed the embryo in agar, a plant gelatin containing nutrients. We hope it will then germinate and grow into a seedling, capable of supporting itself. Hybrid embryos take anywhere from 2 weeks to 4 months to germinate, depending on the species."

If the seed germinates, Wang checks the chromosome pairing in the new plant to see if it is sterile. "It's just like crossing a mare and a jackass to get a mule," says Wang. "The new offspring has the best attributes of its parents but only half the number of chromosomes it needs to reproduce."

To restore fertility in the grass embryos, Wang doubles the plant's chromosomes by treating the seedling with a chemical, colchicine.

One such creation is a new variety of crested wheatgrass, named Hycrest, developed by Wang's coworkers, geneticists Kay H. Asay and Douglas R. Dewey. It was released to plant breeders in 1984.

Hycrest is expected to yield at least 25 percent more grass for livestock and 20 percent more seed for reseeding western rangelands than other popular varieties.—By **Howard Sherman,** ARS.

Richard R-C. Wang is in USDA-ARS Forage and Range Research, Crops Research Laboratory, Utah State University, Logan, UT 84322-6300. ■

Lettuce, Melons, Grapes Travel Cooler

Fresh fruits and vegetables shipped in a new and improved refrigerated van will stay crisp and fresh longer. Because the van provides far better air circulation than conventional refrigerated vans, produce should arrive at its destination no more than 4 to 8 degrees warmer than when it left, according to Agricultural Research Service marketing specialist R. Tom Hinsch.

The uneven cooling that's common in conventional refrigerated vans can drastically shorten shelf life of produce and leave it more susceptible to rotting and similar problems. Asparagus kept at 32°F has a shelf life of 3 weeks; but hold it at 41°—a difference of only 9 degrees—and shelf life shrinks to just a week.

The new van—known as the Advanced Design Perishables Trailer—has been extensively tested using cross-country shipments of lettuce, melons, grapes, oranges, and other fresh fruits and vegetables. Among those who are well pleased with the results is Harold Bradshaw, manager of the California Iceberg Lettuce Research Program, Salinas—one of the many industry contributors to the van experiments. He comments, "This van will provide better air-temperature control than any other van on the road today."

Design features developed and tested by Hinsch and a team of university researchers and industry experts add $1,000 to $2,000 to the base price of a standard refrigerated van but solve these common problems:

- In a conventional refrigerated van, the part of the load that touches the sidewalls absorbs outside heat, even if the walls are insulated.

In the improved van, air from the refrigeration unit is forced through narrow, perforated channels that line the sidewalls—to keep the sides of the load evenly chilled.

- The intake area of the refrigeration unit is located just below the unit, in the front of the van.

The new van has a solid barrier open only a few inches at the bottom so warm air from the bottom of the load can move up through ducts to the intake unit for recooling. Further, unlike the open mesh, this solid wall prevents cool air that's blowing out of the unit from simply taking a shortcut directly to the intake below.

- Not enough air moves through the 1-inch-deep grooves that make up the floor of a conventional van to ensure that the bottom of the load stays cool.

Deeper, 2¼-inch, grooves in the new van provide better circulation underneath the load. —By **Marcia Wood,** ARS.

R. Tom Hinsch, formerly with USDA-ARS Quality Maintenance, Genetics, and Transportation Research Unit, Fresno, CA, is now at the USDA-ARS European Marketing Research Center, Rotterdam, The Netherlands. His mailing address is USDA, Agricultural Research Service, APO, New York, NY 09159. ■

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PATENTS

A Better Way to Shake Trees

This mechanical "shake-and-catch" harvester handles fruit and trees more gently.

The mechanical jaw that grips and shakes the trees is prevented from damaging the trunks as it is withdrawn. A new design for the catching surface above the jaw protects the tree from scrapes as the catcher encircles the tree.

The new catching surface is a neoprene-on-nylon sheet stretched over a tubular steel frame, with tension controlled by springs. When taut, this surface breaks the fall of the fruit, avoiding bouncing damage.

For technical information, contact Donald L. Peterson, USDA-ARS Appalachian Fruit Research Station, Route 2, Box 45, Kearneysville, WV 25430. *Patent Application Serial No. 732,320, "Apparatus to Improve the Operation of a Continuously Moving Harvester for Tree Crops."* ■

Natural Preservatives

The pendulum is swinging back in favor of naturally occurring or derived antimicrobials and preservatives in food, medicine, cosmetics, and other products.

This is because of suspicions about the toxicity and side effects of the synthetic nonfatty compounds that have largely replaced the less potent fatty acid compounds used in the past.

The compounds described in these patents—new compounds derived from vegetable fatty acids and glycolic acid—offer a return to the safety of natural fats but retain potency of the synthetics. Lab tests showed several compounds inhibited the growth of *Staphylococcus aureus, Escherichia coli, Penicillium* mold, and *Candida utilis*—a yeastlike fungus commonly found in the human body.

For technical information, contact August V. Bailey or Gene Sumrell, USDA-ARS Southern Regional Research Center, P.O. Box 19687, New Orleans, LA 70179. *Patent Nos. 4,346,043 and 4,347,378, "Antimicrobial Glycolic Acid Derivates."* ■

Permanent-Press Fabrics That Can Be Dyed

Formaldehyde compounds used to make cotton fabrics permanent press and wrinkle resistant also make the cloth difficult or impossible to dye.

That means the cloth has to be dyed before it is made into a permanent-press garment, restricting manufacturers' choice of colors.

This patent covers several nonformaldehyde finishing agents that make it possible to dye after the cloth is made and treated.

The finishing agent is dissolved in water, or a solvent that is mostly water, along with a mildly acidic substance to serve as a catalyst for the agent's reaction with the fabric.

For technical information, contact National Patent Program Coordinator (address below). *Patent Application Serial No. 774,698, "Dyed Wrinkle-Resistant and Durable-Press Cotton Fabrics."* ■

How to Obtain a License for USDA Patents

A listing of all U.S. Department of Agriculture patents is available on request. If you are interested in applying for a license on a patent or receiving the catalog, write to the Coordinator, National Patent Program, USDA-ARS, Rm. 401, Bldg. 005, Beltsville, MD 20705.

Copies of existing patents may be purchased from the Commissioner of Patents and Trademarks, U.S. Patent and Trademark Office, Washington, DC 20231. Copies of pending patents may be purchased from National Technical Information Service, U.S. Department of Commerce, 5285 Port Royal Road, Springfield, VA 22161.